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Isolation and Prevalence of *Staphylococcus aureus* from raw bovine milk samples in and around Thoothukudi District, Tamilnadu

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ABSTRACT:

In this study, investigation of the presence of *Staphylococcus* and determination of its prevalence and distribution, identification of *Staphylococcus aureus* and determination of their prevalence and distribution and characterization of the isolates in order to determine their ability in synthesizing coagulase from raw bovine milk samples were conducted from Sep 2015 – Mar 2016 in and around Thoothukudi District, Tamilnadu, South India. All the isolates were tested for the production of coagulase to determine their pathogenicity. Comparison of the prevalence of Coagulase Positive *Staphylococci* (CPS) showed a relatively higher CPS prevalence in south zones (78.43%) than other zones (70 – 77%). The high level of *Staphylococcus* isolate found raw milk samples in the present study represent a poor quality and public health risk to the consumer. Hence, raw milk intended for human consumption must be subjected to pasteurization or heat treatment at least equivalent to pasteurization temperature order to guarantee the quality of these highly popular products in these areas in order to decrease the risk of staphylococcal food poisoning.

Key words: Milk samples, CPS, CNS, Gram staining, Microbial pathogens

INTRODUCTION

Milk is an essential part of daily diet for the growing children and expectant mothers. Milk, is a major constituent of the diet, its quality assurance is considered essential to the welfare of a community [1]. Most foods contain viable bacteria unless thoroughly heated or made sterile. Otherwise, it serves as an important medium for transmission of pathogenic organisms to the consumers. Contamination of food products with pathogenic organisms may influence considerably their harmlessness, endanger the health of consumers and decrease shelf quality resulting in food borne infections, intoxications and economic losses from food spoilage [2]. Raw Milk has a varied micro flora arising from several sources, such as exterior surfaces of the animal and the surface of milk handling equipment such as milking machines, pipelines and containers. Therefore, milk is susceptible to contamination by many pathogenic microorganisms, which result in infection [3].

Staphylococci are normal inhabitants of the skin and mucous membranes of animals and humans. Pathogenic strains are usually co-agulase positive and have been found to cause disease in their hosts throughout the world [4]. *Staphylococcus aureus* by far is the most frequent pathogen associated with outbreaks (85.5% of the outbreaks), followed by *Salmonella* (10.1%). Cooked food products and raw milk were most commonly contaminated with food borne pathogens and many of them were resistant to different antibiotics. Milk products are often contaminated with enterotoxigenic strains of *S. aureus* [5]. Presence of enterotoxigenic and antimicrobial resistant strains of *S. aureus* has become remarkably widespread in foods. This requires a better control of food contamination sources and distribution of antimicrobial-resistance organisms [6]. Bearing this in mind, this study was therefore aimed at isolation and prevalence of *Staphylococcus aureus* from raw milk samples and determination of their pathogenicity.

MATERIALS AND METHODS

Collection of milk samples

The study was conducted in and around Thoothukudi district, using stratified random sampling for the dairy farms. The state was divided into four regions; North, South, East and West (NEWS). Random samples were collected from each region (Table 1). The samples were collected in sterile Glass bottles either directly from the udder in cases of individual cows or from the milk bulk tanks or milk containers from milk markets and milk vendors. Samples were then kept in an ice box and transported directly to the Microbiology laboratory, Kamaraj College, Thoothukudi.

Table 1. Physical parameters of *Staphylococcus aureus* from raw bovine milk samples

Zones	Colour	pH
North	Yellowish White	6
South	Paddy straw	5 – 8.5
East	Yellowish White	6,7
West	Yellowish White, Paddy straw	6,7

Enumeration and Isolation of Staphylococci

Five decimal dilutions were prepared by adding 10 ml of the milk sample to 90 ml of peptone water in the first glass bottle resulting in 10^1 dilutions; the process was continued till a dilution of 10^5 is obtained. For each dilution to be plated, aseptically, 1 ml suspension was spread on 3 plates of Baird Parker Agar medium

(0.4, 0.3 and 0.3 ml) of agar plate usi and then incubated for 48 h at 35°C. Plates containing 20 - 200 colonies with appearance of *Staphylococci* were selected. Five to seven colonies from each plate were selected and inoculated into 5 ml brain heart infusion broth and then incubated for 24 h at 37°C. 0.1 ml of brain heart infusion broth was added to a test tube containing 0.3 ml of rabbit plasma. The tubes were incubated for 4 - 6 h at 37°C. The isolates were classified as CPS or CNS using coagulase test [7].

Identification of Staphylococcus aureus

Gram stain

All suspected cultures of *Staphylococcus* species were subjected to Gram's stain. The Gram-stained smears from typical colonies that showed Gram-positive cocci occurring in bunched, grapelike irregular clusters were considered as presumptive *Staphylococcus* species.

Catalase test

Pure culture of the isolates were picked using a sterile loop from the agar slant and mixed with a drop of 3% H_2O_2 on a clean glass slide. If the organism was positive, bubbles of oxygen were liberated within a few seconds and the catalase negative isolates did not produce bubbles. The catalase positive cocci were considered as *staphylococci*.

Mannitol salt agar

The colonies that were identified by Gram-staining and catalase test as *Staphylococcus* were streaked on Mannitol Salt Agar (MSA) plates and incubated at 37°C and examined after 24-48 h for growth and change in the colour of the medium. The presence of growth and change of pH in the media (red to yellow colour) were regarded as confirmative identification of the salt tolerant *Staphylococci*. Phenol red pH indicator detected the acidic metabolic product of mannitol. Fermentation of mannitol by *S. aureus* causes yellow discolouration of the medium.

Coagulase test

The tube coagulase test was performed in sterile tubes by adding 0.5 ml of selected isolates of *Staphylococcus* grown on Tryptone Soya Broth (TSB) at 37°C for 24 h to 0.5 ml of citrated rabbit plasma. After mixing by gentle rotation, the tubes were incubated at 37°C along with a negative control tube containing a mixture of 0.5 ml of sterile TSB and 0.5 ml of rabbit plasma. Clotting was evaluated at 30 min intervals for the first 4 h of the test and then after 24 h incubation. The reaction was considered positive if any degree of clotting from a loose clot to a solid clot that is immovable when the tube is inverted was visible within the tube and no degree of clotting would be taken as negative.

RESULTS AND DISCUSSION

A range of physical parameters were studied after collecting the sampling of milk from different villages and surrounding areas in Thoothukudi district. Table 1 shows the color and the pH of the samples analyzed in the test which had a pH range between 5 and 8.5. Out of 204 samples analyzed, 154 samples were found yellowish white, 36 samples were white, 10 samples were light yellowish white and remaining 4 samples were deep yellowish white in color. These findings agreed with the reports of Judkins and Mack [8], who reported that normal milk has a yellowish white color due to the presence of fat, casein and the presence of small amount of colouring matter. These differences in color may be due to the differences in nature of feed consumption or the breed of cow or the fat and solid contents of the milk [9].

Out of the 204 raw milk samples 64.70% (132/204) resulted contaminated with *Staphylococcus aureus* (Table 2).

Table 2. Prevalence of *Staphylococcus aureus* from raw bovine milk samples

Zones	Examined (samples)	Positive Strains for *S.aureus*	Prevalence (%)
North	4	4	100
South	164	102	62.19
East	16	10	62.5
West	20	16	80
Total	204	132	64.70

The frequency of isolation of *Staphylococcus* varied between zone types and ranged from 62 - 100%. The prevalence of *S. aureus* was 100% (4/4), 62.19% (102/164), 62.5% (10/16) and 80% (16/20) from North, South. East and West zones, respectively. All isolates were characterized in order to determine their ability in synthesizing coagulase. Raw milk samples yielded an overall prevalence of 64.70% (132/204) of *S.aureus*. Specifically the prevalence of Coagulase positive *S.aureus* was 77.27 % (102/132) and negative strains was 22.72% (30/132) (Table 3).

The milking process, especially the equipment associated with it introduces the greatest proportion of microorganism in cow milk [10]. According to Aumaitre [11] the health of the dairy herd, milking and pre storage conditions are also basic determinants of milk quality. Bacteria may enter milk through the udder and most of the organisms in raw milk are contaminants from the external surface of udder, milking utensils and handlers [12]. Various types of equipment and utensils, such as milking machines, pails, cans and milk churns are used in handling milk on the farm. In order to reduce contamination of milk, utensils used for milking should be rinsed, cleaned

using detergent and disinfected immediately after use [13].

Table 3. Proportional distribution coagulase positive *S.aureus* Strains in raw milk samples

Zones	Examined	Prevalence (%)	Coagulase test (%)	
			Positive	Negative
North	4	100	75 (3)*	25(1)*
South	164	62.19	78.43 (80)*	21.56 (22)*
East	16	62.5	70 (7)*	30 (3)*
West	20	80	75 (12)*	25 (4)*
Total	204	132	77.27 (102)	22.72 (30)

* No of samples

This could be attributed to the cumulative effects of milk contamination at different critical points. Additionally, handling of milk in different plastic containers and the use of sieves may cause contamination of milk. Plastic containers have characteristics that make them unsuitable for milk handling. Plastic containers scratch easily and provide hiding places for bacteria during cleaning and sanitization and plastic containers is poor conductor of heat and hence will hinder effective sanitization by heat [14]. Also, the number of personnel working at MCCs was higher which might have contributed to milk contamination.

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